CLAIMS

What is claimed:

1. A method for isolating a from a target plant species a target polynucleotide encoding a target polypeptide comprising a conserved region exhibiting at least 70% sequence identity to a conserved region of template polypeptide that is encoded by a template polynucleotide from a template plant species, comprising:

(a) identifying an amino acid sequence of a conserved region in the template polypeptide;

(b) generating an oligonucleotide comprising a sequence wherein the sequence or its reverse complement comprises at least four codons that encode a portion of the amino acid sequence of (a), wherein

(i) the sequence of the first and second positions of at least three of the codons is the same as the corresponding nucleotides in nucleotides in the template polynucleotide;

(ii) the nucleotide at the third position of the codons of (i) is the nucleotide of the third position of the most preferred codon of the target plant class for the desired amino acid;

(c) contacting the oligonucleotide with a composition comprising the target polynucleotide under conditions that permit hybridization of the oligonucleotide to the target polynucleotide to form a duplex; and

(d) isolating the duplex.

2. The method of claim 1, wherein the oligonucleotide does not contain a homopolymer of more than four guanine or cytosine residues.

3. The method of claim 1, wherein the oligonucleotide does not contain a homopolymer of more than four residues.

4. The method of claim 1, wherein the oligonucleotide of step (b) wherein the sequence or its reverse complement further comprises at least one codon wherein

(i) the sequence of the first and second position of the codon is the same as the corresponding nucleotides in the template polynucleotide;

(ii) the sequence of the third position of the codon of step (I) is the same as the nucleotide of the third position of the second most preferred codon of the target plant species for the desired amino acid; and

(iii) the oligonucleotide is not degenerate.

5. The method of claim 1, wherein the target polynucleotide is from a monocot plant and the template polynucleotide is from a dicot plant.

6. The method of claim 4, wherein the template polynucleotide is from Arabidopsis.

7. The method of claim 5, wherein the third position of each codon is either a guanosine or cytosine.

8. The method of claim 2, wherein both the template and target polynucleotides are from dicot plants.

9. The method of claim 8, wherein the template polynucleotide is from Arabidopsis.

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10. The method of claim 9, wherein the third position of each codon is either an adenosine or thymidine.

11. The method of claim 1, wherein the template polynucleotide is from a monocot plant and the target polynucleotide is from a dicot plant.

12. The method of claim 1, wherein both the template and target polynucleotides are from monocot plants.

13. The method of claim 11 or 12, wherein the template polynucleotide is from corn.

14. The method of claim 12, wherein the target polynucleotide is from corn.

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15. The method of claim 1, wherein step (a) comprises aligning polynucleotides of plants within a family and identifying a portion of the template polynucleotide that exhibits at least 70% sequence identity to a portion of a polynucleotide from a plant of a genus closely related to the plant from which the template polynucleotide originates.

16. The method of claim 1, wherein step (a) comprises identifying the primary sequence in a region of the template polypeptide sequence that forms a secondary structure.

17. The method of claim 16, wherein the secondary structure is a helix or a beta sheet.

18. The method of claim 1, wherein the conserved region is a motif or functional domain.

19. The method of claim 1, wherein step (a) comprises identifying the primary sequence in a region of the template polypeptide that is repeated.

20. The method of claim 1, wherein the oligonucleotide comprises from 6 to 11 codons.

21. The method of claim 1, wherein step (c) further includes contacting the composition comprising the target polynucleotide with a second oligonucleotide, wherein the second oligonucleotide is a degenerate oligonucleotide encoding a second portion of the conserved region.

22. A method of isolating a target polynucleotide encoding a conserved region in a template polypeptide encoded by a template polynucleotide comprising:

(a) identifying the amino acid sequence of the conserved region in the template polypeptide;

(b) generating an oligonucleotide comprising a sequence wherein the sequence or its reverse complement comprises at least four codons that encode a portion of the conserved region of step (a), wherein

(i) the sequence of the first and second positions of at least three codons is the same as the corresponding nucleotides in the template polynucleotide;

(ii) the nucleotide of the third position of those six codons is the same nucleotide in the third position of the most preferred codon of the target plant species for the desired amino acid;

(iii) the oligonucleotide does not comprise homopolymers of more than four nucleotides; and

(iv) the oligonucleotide is not degenerate;

(c) contacting the oligonucleotide with a composition comprising the target polynucleotide under conditions that permit hybridization of the oligonucleotide to the target polynucleotide to form a duplex;

(d) contacting the duplex of step (c) with a thermostable polymerase under conditions to elongate the oligonucleotide of step (b); and

(e) isolating the elongation product of step (d).

23. A method for identifying a target polynucleotide encoding a conserved region in a template polypeptide encoded by a template polynucleotide comprising:

(a) identifying the amino acid sequence of the conserved region in the template polypeptide;

(b) generating an oligonucleotide comprising a sequence wherein the sequence or its reverse complement comprises four codons that encode a portion of the conserved region of step (a), wherein

(i) the sequence of the first and second positions of at least three codons is the same as the corresponding nucleotides in the template polynucleotide;

(ii) the nucleotide of the third position of those six codons is the same nucleotide in the third position of the most preferred codon of the target plant species for the desired amino acid;

(iii) the oligonucleotide does not comprise homopolymers of more than four nucleotides; and

(iv) the oligonucleotide is not degenerate;

(c) contacting the oligonucleotide with a composition comprising the target polynucleotide under conditions that permit hybridization of the oligonucleotide to the target polynucleotide to form a duplex;

(d) contacting the duplex of step (c) with a thermostable polymerase under conditions to elongate the oligonucleotide of step (b); and

(e) determining the nucleotide sequence of the elongation product of step (d).

24. A method of isolating a target polynucleotide encoding a polypeptide of a conserved region in a template polypeptide encoded by a template polynucleotide, comprising:

(a) identifying the amino acid sequence of the conserved region in the template polypeptide;

(b) generating a first oligonucleotide comprising a sequence wherein the sequence or its reverse complement comprises four codons that encode a first portion of the conserved region of step (a), wherein

(i) the sequence of the first and second position of at least three codons is the same as the corresponding nucleotides in the template polynucleotide;

(ii) the nucleotide of the third position of the codons of step (i) is the same as the nucleotide in the third position of the most preferred codon of the target plant species for the desired amino acid;

(iii) the oligonucleotide does not comprise homopolymers of more than four nucleotides; and

(iv) the oligonucleotide is not degenerate;

(c) generating a second oligonucleotide wherein its sequence or its reverse complement comprises four codons that encode a second portion of the conserved region of step (a), wherein

(i) the sequence of the first and second position of at least three codons is the same as the corresponding position in the template polynucleotide;

(ii) the nucleotide of the third position of those codons is the same as the nucleotide of the third position of the most preferred codon of the target plant species for the desired amino acid;

(iii) the oligonucleotide does not comprise homopolymers of more than four nucleotides; and

(iv) the oligonucleotide is not degenerate;

(d) contacting the first and second oligonucleotides with a composition comprising the target polynucleotide under conditions that permit hybridization of at least one of the oligonucleotides and the target polynucleotide to form a duplex;

(e) contacting the duplex of step (d) with a thermostable polymerase under conditions to elongate the at least one hybridized oligonucleotide;

(f) generating a strand complementary to the elongation product of step (e); and

(g) isolating the product of step (d).

25. The method of claim 24, wherein the two oligonucleotide sequences or their reverse complements encode portions of the conserved region of step (a) that are separated by at least 30 amino acids.

26. The method of claim 24, wherein the two oligonucleotide sequences or their reverse complement encode between 6 to 11 amino acids of the conserved region of step (a).

27. The method of claim 24, wherein the product of step (f) is inserted into a vector.

28. A method for identifying a target polynucleotide encoding a polypeptide of a conserved region in a template polypeptide encoded by a template polynucleotide, comprising:

(a) identifying the amino acid sequence of the conserved region in the template polypeptide;

(b) generating a first oligonucleotide comprising a sequence wherein the sequence or its reverse complement comprises four codons that encode a first portion of the conserved region of step (a), wherein

(i) the sequence of the first and second position of at least three codons is the same as the corresponding nucleotides in the template polynucleotide;

(ii) the nucleotide of the third position of the codons of step (i) is the same as the nucleotide in the third position of the most preferred codon of the target plant species for the desired amino acid;

(iii) the oligonucleotide does not comprise homopolymers of more than four nucleotides; and

(iv) the oligonucleotide is not degenerate;

(c) generating a second oligonucleotide wherein its sequence or its reverse complement comprises four codons

that encode a second portion of the conserved region of step (a), wherein

(i) the sequence of the first and second position of at least three codons is the same as the corresponding position in the template polynucleotide;

(ii) the nucleotide of the third position of those codons is the same as the nucleotide of the third position of the most preferred codon of the target plant species for the desired amino acid;

(iii) the oligonucleotide does not comprise homopolymers of more than four nucleotides; and

(iv) the oligonucleotide is not degenerate;

(d) contacting the first and second oligonucleotides with a composition comprising the target polynucleotide under conditions that permit hybridization of at least one of the oligonucleotides and the target polynucleotide to form a duplex;

(e) contacting the duplex of step (d) with a thermostable polymerase under conditions to elongate the at least one hybridized oligonucleotide;

(f) generating a strand complementary to the elongation product of step (e); and

(g) determining the nucleotide sequence of the product of step (f).

29. A method for selecting a nucleotide sequence of an oligonucleotide primer for a polymerase chain reaction comprising:

(a) selecting a nucleotide sequence encoding a desired amino acid sequence from a template organism, or the complement thereof;

(b) selecting for the nucleotide of the third position of each codon the preferred codon for a target organism, provided said nucleotide is guanine or cytosine;

(c) if the nucleotide of the third position of the preferred codon is adenine or thymine, then substituting either a guanine or cytosine, selecting guanine or cytosine to avoid introducing a poly-guanylate or polycytidylate sequence of more than four residues;

wherein said desired amino acid sequence is encoded by one reading frame, or a portion thereof, of the nucleotide sequence of said primer or the complement thereof.

30. A method for preparing an oligonucleotide primer for a polymerase chain reaction comprising:

(a) selecting a nucleotide sequence encoding a desired amino acid sequence from a template organism, or the complement thereof;

(b) selecting for the nucleotide of the third position of each codon the preferred codon for a target organism, provided said nucleotide is guanine or cytosine;

(c) if the nucleotide of the third position of the preferred codon is adenine or thymine, then substituting either a guanine or cytosine, selecting guanine or cytosine to avoid introducing a poly-guanylate or polycytidylate sequence of more than four residues; and

(d) synthesizing said oligonucleotide primer, wherein said desired amino acid sequence is encoded by one reading frame, or a portion thereof, of the nucleotide sequence of said primer or the complement thereof.

31. A method for cloning a nucleic acid comprising:

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(a) selecting an upstream nucleotide sequence encoding a first desired amino acid sequence from a template organism and a downstream nucleotide sequence encoding a second desired amino acid sequence;

(b) for each of said upstream and downstream nucleotide sequences, selecting for the nucleotide of the third position of each codon the preferred codon for a target organism, provided said nucleotide is guanine or cytosine;

(c) if the nucleotide of the third position of the preferred codon is adenine or thymine, then substituting either a guanine or cytosine, selecting guanine or cytosine to avoid introducing a poly-guanylate or polycytidylate sequence of more than four residues.

32. The method of claim 31, further comprising:

(d) synthesizing an upstream oligonucleotide primer, or a portion thereof according to steps (b) and (c).

33. The method of claim 32, further comprising:

(e) performing a polymerase chain reaction using said upstream and downstream primers and a template comprising a nucleic acid sample obtained from said target organism.

34. The method of claim 33, further comprising:

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(f) using the product of said polymerase chain reaction of step (e) as a probe to screen a library prepared from nucleic acids obtained from said target organism.

35. The method of claim 33, further comprising:

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(f') inserting the product of the polymerase chain reaction of step (e) into a vector.

36. The method of any one of claims 30-35, wherein said template organism is a dicot and said target organism is a monocot or wherein said template organism is a monocot and said target organism is a dicot.

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37. A method for isolating a target polynucleotide encoding a target polypeptide comprising a conserved region of a template polypeptide that is encoded by a template polynucleotide, comprising:

(a) identifying an amino acid sequence of a conserved region in the template polypeptide;

(b) generating an oligonucleotide comprising a sequence wherein the sequence or its reverse complement comprises at least four codons that encode a portion of the amino acid sequence of (a), wherein

(i) the sequence of the first and second positions of at least three of the codons is the same as the corresponding nucleotides in nucleotides in the template polynucleotide;

(ii) the nucleotide at the third position of the codons of (i) is the nucleotide of the third position of the most preferred codon of the target plant species for the desired amino acid;

(c) contacting the oligonucleotide with a composition comprising the target polynucleotide under conditions that permit hybridization of the oligonucleotide to the target polynucleotide to form a duplex; and

(d) generating a single strand polynucleotide.

38. A method for isolating a from a target plant species a target polynucleotide encoding a target polypeptide comprising a conserved region exhibiting at least 70% sequence identity to a conserved region of template polypeptide that is encoded by a template polynucleotide from a template plant species, comprising:

(a) identifying an amino acid sequence of a conserved region in the template polypeptide;

(b) generating an oligonucleotide comprising a sequence wherein the sequence or its reverse complement comprises at least four codons that encode a portion of the amino acid sequence of (a), wherein

(i) the sequence of the first and second positions of at least three of the codons is the same as the corresponding nucleotides in nucleotides in the template polynucleotide;

(ii) the nucleotide at the third position of the codons of (i) is the nucleotide of the third position of the most preferred codon of the plant family of target plant species for the desired amino acid;

(c) contacting the oligonucleotide with a composition comprising the target polynucleotide under conditions that permit hybridization of the oligonucleotide to the target polynucleotide to form a duplex; and

(d) isolating the duplex.

39. A method for isolating a from a target plant species a target polynucleotide encoding a target polypeptide comprising a conserved region exhibiting at

least 70% sequence identity to a conserved region of template polypeptide that is encoded by a template polynucleotide from a template plant species, comprising:

(a) identifying an amino acid sequence of a conserved region in the template polypeptide;

(b) generating an oligonucleotide comprising a sequence wherein the sequence or its reverse complement comprises at least four codons that encode a portion of the amino acid sequence of (a), wherein

(i) the sequence of the first and second positions of at least three of the codons is the same as the corresponding nucleotides in nucleotides in the template polynucleotide;

(ii) the nucleotide at the third position of the codons of (i) is the nucleotide of the third position of the most preferred codon of the plant genera for the target plant species for the desired amino acid;

(c) contacting the oligonucleotide with a composition comprising the target polynucleotide under conditions that permit hybridization of the oligonucleotide to the target polynucleotide to form a duplex; and

(d) isolating the duplex.

40. A method for isolating a from a target plant species a target polynucleotide encoding a target polypeptide comprising a conserved region exhibiting at least 70% sequence identity to a conserved region of template polypeptide that is encoded by a template polynucleotide from a template plant species, comprising:

(a) identifying an amino acid sequence of a conserved region in the template polypeptide;

(b) generating an oligonucleotide comprising a sequence wherein the sequence or its reverse complement comprises at least four codons that encode a portion of the amino acid sequence of (a), wherein

(i) the sequence of the first and second positions of at least three of the codons is the same as the corresponding nucleotides in nucleotides in the template polynucleotide;

(ii) the nucleotide at the third position of the codons of (i) is the nucleotide of the third position of the most preferred codon of the plant species of the target plant species for the desired amino acid;

(c) contacting the oligonucleotide with a composition comprising the target polynucleotide under conditions that permit hybridization of the oligonucleotide to the target polynucleotide to form a duplex; and

(d) isolating the duplex.

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